ANB032, an Investigational BTLA Agonist Antibody, Reduced T Cell Proliferation, Inflammatory Cytokine Secretion, and Prolonged Survival in a Mouse Model of Graph versus Host Disease

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ABSTRACT

BTLA is a co-inhibitory checkpoint receptor and the interaction with its ligand, herpesvirus entry mediator (HVEM), induces inhibitory signals that regulate the activation of T cells, B cells, and dendritic cells. ANB032 is an investigational BTLA agonist IgG4 antibody that does not compete with the binding of BTLA to HVEM. ANB032 has been shown to reduce T cell proliferation and secretion of inflammatory cytokines (Th1, Th2, Th17, Th22) in AD patient-derived peripheral blood mononuclear cells (PBMCs) in vitro. ANB032 also modulates DC function while inducing regulatory T cells in vitro. The in vivo efficacy and immune regulatory effects of ANB032 were evaluated in a humanized mouse model of GvHD, where human PBMCs were adoptively transferred to irradiated NSG mice. ANB032 was compared to two reference BTLA agonists to determine the contribution of sparing the HVEM/BTLA binding interaction and Fc receptor engagement. Both reference BTLA agonist antibodies (Ref1 and Ref2) lacked FcR engagement due to mutations in the Fc domain. In addition, Ref1 spared the HVEM binding to BTLA, whereas Ref2 blocked HVEM/BTLA binding. In the humanized GvHD mouse model, ANB032 reduced T cell expansion, reduced the levels of inflammatory cytokines in plasma, and demonstrated greater in vivo efficacy on key endpoints, including prolonged survival, maintenance of body weight and reduced disease activity index (DAI), compared to both reference BTLA agonists. These data suggest the importance of sparing the HVEM/BTLA binding interaction together with the requirement of Fc receptor engagement for optimizing potency and the potential therapeutic benefit of BTLA agonism with ANB032 in the treatment of inflammatory diseases such as atopic dermatitis (AD). This study, additional in vitro data, and results from a Phase 1 study in healthy volunteers support the ongoing double-blind, placebo-controlled, global Phase 2b study of ANB032 in moderate-to-severe AD (NCT05935085).

BACKGROUND

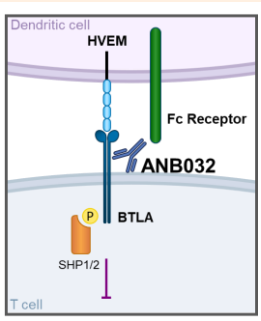
- B and T Lymphocyte Attenuator (BTLA) belongs to a family of co-inhibitory checkpoint receptors critical for immune system regulation, and is expressed preferentially on activated T cells, B cells, and dendritic cells (DCs), which are key contributors to inflammatory diseases
- ANB032 is an investigational BTLA agonist antibody that has been shown to reduce activated T cell proliferation, reduce inflammatory cytokine secretion (Th1, Th2, Th17, Th22), and modulate DC function while inducing Tregs[1]
- Atopic dermatitis (AD) is a systemic, heterogenous inflammatory disease with pathogenesis driven by Th1, Th2, Th17, Th22, and DCs, both in the skin and in the periphery therefore ANB032 may potentially play a role in reducing inflammation and restoring immune homeostasis
- ANB032 and two reference BTLA agonists were evaluated to determine the effect of potency and the blocking of the natural HVEM/BTLA binding interaction in a humanized mouse model of GvHD (**Table 1**)
- The effect of BTLA agonism on inflammatory cytokine secretion was investigated with AD patient-derived peripheral blood mononuclear cells (PBMCs) in vitro

Antibody characteristics of ANB032, Ref1 & Ref2

	ANB032	Ref1	Ref2
HVEM Sparing	✓	✓	✗
FcR Engagement	✓	✗	✗

Table 1. Description of BTLA agonists

Proposed Mechanism of Action for ANB032



ANB032: IgG4 antibody (non-depleting)

- Binds to BTLA on membrane proximal epitope
- Fc receptor binding profile contributes to differentiated potency
- Non-blocking of HVEM engagement

ANB032's agonist signal modulates immune cells

- Inhibits activated T cell proliferation
- Reduces inflammatory cytokine secretion
- Modulates DC function, including inducing Tregs

METHODS

Humanized Mouse Model GvHD

- NOD-skid IL-2rγnull mice were engrafted with human PBMCs one day prior to 4-week BiW dosing with BTLA agonist antibodies or isotype control (**Figure 1**)
- Blood was collected for fluorescence-activated cell sorting (FACS) analysis at study day 18 midpoint (determined when isotype control animals exhibited GvHD manifestations) (**Figure 2**)
- Clinical observations of survival, body weight (BW), and disease activity index (DAI: fur skin, posture, and activity) were collected twice weekly (BiW) (**Figure 3**)
- Inflammatory cytokines levels were measured from plasma collected at midpoint (SD18) (**Figure 4**)

AD Patient-Derived PBMCs

- PBMCs from AD donors were treated with anti-CD3 and anti-CD28 in vitro for 72h to stimulate T cell proliferation, in the presence of ANB032 or isotype control mAb. Supernatants were collected for analysis by MSD to measure inflammatory cytokine secretion (**Figure 5**)

Humanized Mouse Model of GvHD

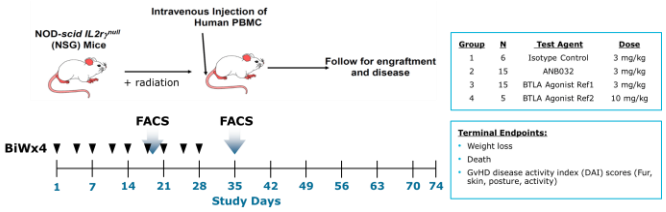


Figure 1. Schematic of humanized mouse model of GvHD with study details

RESULTS

ANB032 Reduced Human T Cell Expansion

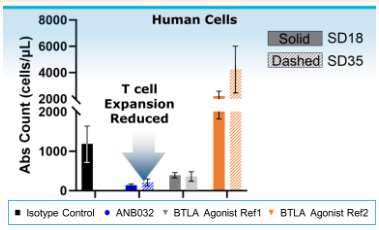


Figure 2. Quantification of circulating human CD45+ cells in whole blood of mice surviving at Study Day 18 (SD18 solid fill) and 35 (SD35 dashed fill)

ANB032 Resulted in Prolonged Survival and Reduced Disease Activity Index

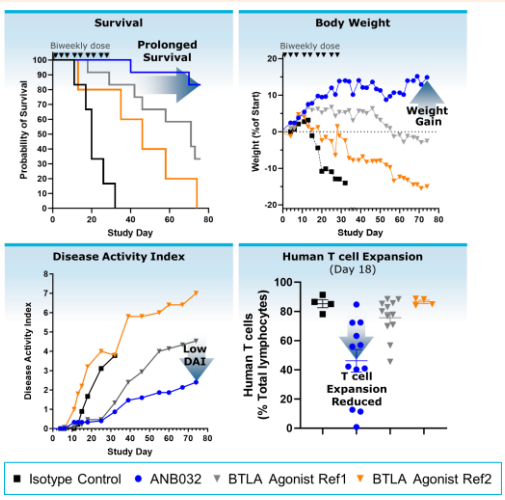


Figure 3. Evaluation of clinical observations (survival, body weight, and disease activity index) and human T cell expansion

ANB032 Reduced Inflammatory Cytokines

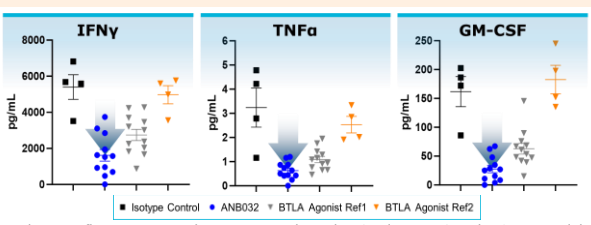


Figure 4. Plasma inflammatory cytokines were evaluated at Study Day 18 in the GvHD model

ANB032 Reduced Th1, Th2, Th17 & Th22 Cytokine Secretion in AD Patient-derived PBMCs in Vitro

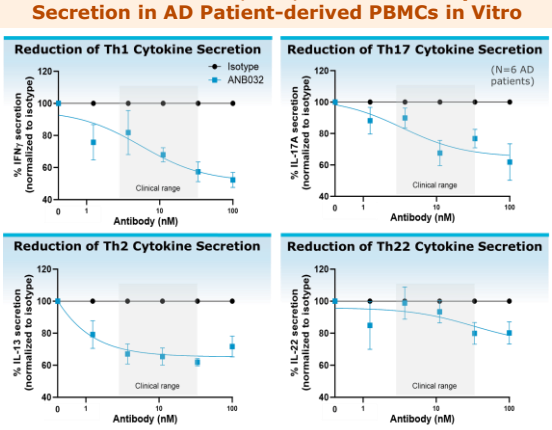


Figure 5. In vitro evaluation of IFN-γ, IL-17A, IL-13, and IL-22 secretion in AD patient-derived PBMCs

CONCLUSIONS

- In a humanized mouse model of GvHD, ANB032:
  - Reduced T cell expansion and inflammatory cytokines
  - Demonstrated superior in vivo efficacy on key endpoints, including prolonged survival, maintained BW and an overall reduced DAI, compared to reference BTLA agonist antibodies
  - Demonstrated the importance of HVEM sparing (not blocking HVEM), the natural BTLA ligand, resulting in enhanced potency when compared to an HVEM blocking anti-BTLA reference
- ANB032 reduced Th1, Th2, Th17 and Th22 inflammatory cytokine secretion from atopic dermatitis patient-derived PBMCs
- ANB032 is being evaluated in an ongoing Phase 2 study in moderate-to-severe AD (NCT05935085)

REFERENCES

1. Muench, et al. Presented at AAD 2024.

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1. This research was supported by Anaptys
2. Author disclosures: All authors are employees of Anaptys
3. This research was previously presented at American Association of Immunology, May 2024